

MSU 4.1-406
Appl. No. 09/082,112
Amdt. dated March 20, 2006
Reply to Office Action of December 21, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions,
and listings, of claims in the application:

LISTING OF CLAIMS

Claims 1-15 (Cancelled).

16. (Currently Amended):

A method for treatment of an infection caused by
Pythium insidiosum ~~of Pythiosis~~ in human patients having
the Pythiosis which comprises:

(a) providing a vaccine containing a mixture of
(1) mixed intracellular proteins and (2) mixed
extracellular proteins of *Pythium insidiosum* in a sterile
aqueous solution, wherein the mixed intracellular
proteins, which consist essentially of the intracellular
proteins removed as a supernatant separated from disrupted
cells of the *Pythium insidiosum* grown in a culture medium,
and the mixed extracellular proteins, which consist

essentially of proteins removed from the culture medium for growing the *Pythium insidiosum* the mixed intracellular proteins and the mixed extracellular proteins have by having been precipitated together from the culture medium with acetone, separated and then mixed with water and the mixture has been dialyzed to remove low molecular weight components less than 10,000 MW; and

(b) vaccinating human the patient with the vaccine.

17. (Previously Presented):

The method of Claim 16 wherein vaccinating the patient with the vaccine is subcutaneous.

18. (Currently Amended):

A method for ~~the treatment of~~ an infection caused by *Pythium insidiosum* ~~of Pythiosis~~ in a mammal having ~~the~~ Pythiosis which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium; and

(2) mixed extracellular proteins, which consist essentially of proteins removed from the culture medium separated from the cells of the *Pythium insidiosum*;

wherein the admixture of intracellular proteins and extracellular proteins has been precipitated ~~from the culture medium~~ with acetone, separated and admixed with water and then has been dialyzed to remove low molecular

weight components less than 10,000 MW to produce the vaccine; and

(b) vaccinating the mammal with the vaccine.

19. (Previously Presented):

The method of Claim 18 wherein the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant to provide the mixed extracellular proteins of (a)(2) and then disrupting the killed cells in sterile water and removing the disrupted cells from the sterile water containing the mixed intracellular proteins to provide the mixed intracellular proteins of (a)(1) in a second supernatant, combining the first and second supernatants, precipitating the proteins with the acetone, resuspending the precipitated proteins in sterile water, and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW.

20. (Original):

The method of Claim 18 wherein the cells have been disrupted by sonication.

21. (Previously Presented):

The method of Claim 18 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

22. (Original):

The method of any one of Claims 19, 20 or 21 wherein the culture medium is Sabouraud's dextrose broth.

23. (Original):

The method of Claim 19 wherein the cells are killed with thimersol.

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24. (Previously Presented) :

The method of Claim 19 wherein the disrupted cells are removed from the sterile water containing the mixed intracellular proteins by centrifugation to provide the mixed intracellular proteins of (a)(1) in the second supernatant.

Claims 25-27. (Cancelled).